The Urinary Elimination of Nicotinamide Methochloride by Man

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The development of a method for the estimation of nicotinamide methochloride in urine by Coulson, Ellinger & Holden (1944) provided a means of studying its quantitative elimination in human urine under a variety of conditions. A preliminary method (Coulson, Ellinger, Glock & Platt, 1942) which gave only comparative but not quantitative results had been used before the chemical nature of the pigments involved was elucidated by Huff & Perlzweig (1943), Ellinger & Coulson (1943) and Coulson & Ellinger (1943). This paper is concerned with the elimination of nicotinamide methochloride by a number of subjects under normal conditions and under the influence of some extrinsic factors. The effect of the intake of nicotinamide and several related compounds on the elimination of the methylated derivative was studied, and an attempt was made to determine the state of saturation in man with respect to nicotinamide.

EXPERIMENTAL METHODS

Method of determination of nicotinamide methochloride

Urine was collected at known intervals and the volume per hour was calculated. In the preliminary experiments 10 ml. were filtered through 1 g. columns of Decalco which were then washed with 50 ml. of water and eluted with 14 ml. of 25% (w/v) KCl. The eluates were divided into two equal parts, one of which was made alkaline with 1 ml. of 15% (w/v) NaOH. Both were shaken with 2 ml. of isobutanol for 5 min. The isobutanol extracts were transferred to small non-fluorescent test-tubes, dried with anhydrous Na_2SO_4 and their fluorescence intensity examined in the 366 m μ line of a high-pressure mercury lamp. The fluorescence intensity was given relative arbitrary units.

In the earlier experiments losses of nicotinamide methochloride occurred through incomplete adsorption due to high urinary electrolyte concentration, and the visual fluorimetry of the isobutanol extracts gave too low readings when the nicotinamide methochloride concentration was high. A conversion of these units into milligrams is, therefore, impossible. In the later experiments the quantitative method by Coulson *et al.* (1944) was used and the results were expressed in mg.

Collection of urines

Urine samples taken at frequent intervals throughout 24 hr. or complete pooled 24 hr. samples were collected from 24 subjects. The early experiments showed that the output of nicotinamide methochloride varied throughout

the day. Consequently first morning samples were chosen, since these could be expected to be least affected by extrinsic factors such as food, alcohol in drinks, physical exercise, etc., during the period of collection. Seven-hour samples collected between 10 a.m. and 5 p.m. after ingestion of 100 mg. nicotinamide were later used for comparative experiments.

RESULTS

Normal daily elimination curves

Complete 24 hr. elimination curves were obtained from three subjects leading a uniform sedentary life, one normal male (1), one normal female (2) and one diabetic male (3) balanced by diet and insulin. The normal subjects were living on war-time diet, but the diabetic subject received increased rations of meat, butter, margarine, cheese and milk.

Fig. 1. Normal daily elimination curve of nicotinamide methochloride of subjects (1) —, (2) ----, and (3) - - - -.

The daily elimination of nicotinamide methochloride was also determined for 21 normal young airmen for 2 days by the earlier method. The results of the investigation of the three subjects is shown in Fig. 1. Each curve shown represents the average elimination on 3 normal days. The absolute daily output varies considerably.

Subject (1) eliminated an average of 7.6 mg., subject (2) 6.7 mg., and subject (3) 4.0 mg. The three curves, however, have the same shape, with a medium level in the early morning, a peak in the forenoon, a fall below the night level in the early afternoon, a decline to a minimum in the evening and a gradual rise during the night. The elimination curves gained from the 21 normal young airmen showed the same general shape.

There is some indication that the total daily output was subject to seasonal changes although the evidence at hand is not conclusive.

Effect of extrinsic factors

Food. As far as could be ascertained ordinary diet did not influence elimination of the methochloride. The morning rise is observed in fasting subjects as well as in those who had eaten breakfast. Liver in small amounts produced no significant increase in the output. The ingestion of 30 g. of dried yeast (*Torula utilis*, kindly supplied by Dr A. C. Thaysen) produced a slight increase.

Alcohol. Nicotinamide and related compounds are of value in the treatment of pellagra, and continuous and excessive intake of alcohol is associated with the onset of alcoholic pellagra; this suggested the study of the effect of ingestion of large amounts of alcohol in a short time on the elimination of nicotinamide methochloride following ingestion of nicotinamide or a related compound. For this purpose a fairly high output of nicotinamide methochloride was induced in subjects (1) and (3) by the daily intake of nikethamide in doses equivalent to 2.5-5.0 mg. nicotinamide/kg. body weight, and then 1 g. of alcohol/kg. body weight diluted with water to a 25 % solution was drunk in the space of 30 min. Neither subject was accustomed to large doses of concentrated alcoholic drinks. The daily nikethamide intake was continued for 4 weeks and alcohol was taken three times at intervals of about 10 days. In both subjects the alcohol imbibition was always followed by a temporary increase in the nicotinamide methochloride elimination and then by a rapid decline. This fall lasted 2-4 days after the first alcohol dosage, 3-6 days after the second, and for more than a week after the third.

Work. Observations on pellagrins in Yugoslavia and Egypt had shown that pellagrous symptoms were precipitated only in those engaged in hard work, while the rest of the population living on the same diet but leading a sedentary life showed only pre-pellagrous symptoms (Ellinger & Dojmi, 1935; and Ellinger, Hassan & Taha, 1937a); in addition, milder cases of pellagra were cured by rest alone without any change of diet after being admitted to hospital (Ellinger *et al.* 1937b). These observations suggested that work may play a considerable role in the metabolism of nicotinamide and consequently influence the urinary elimination of nicotinamide methochloride. Some indication that this might be the case was gained from the following experiment. Daily administration of small amounts of nicotinamide to the same subject produces a consistent rise of the same height and duration. One mg. of nicotinamide/kg. was taken by subject (3) each day before, during, and on the days after a 3-day period of unusually intense physical and mental strain. The elimination on the day before the strain was 450 arbitrary units; this decreased to 75 units on the first day after the strain, and the two following

days showed a gradual rise to 321 units and 432 units respectively.

Effect of administration of nicotinamide and related compounds

The effect of a number of compounds on the elimination of nicotinamide methochloride has been studied. Nicotinamide, nicotinic acid, nikethamide and nicotinic acid-mono-ethyl-amide were found to be active, whereas trigonelline, pyridoxine, nicotinonitrile, aneurin or riboflavin did not increase the output of the methylated derivative.

The total elimination of nicotinamide methochloride for subjects (1), (2) and (3) after ingestion of various amounts of nicotinamide, nicotinic acid and nikethamide appears in Table 1.

Some indication of the rate of elimination was obtained by making determinations at 13, 37, 61 and 85 hr. after ingestion of nicotinamide, etc. The examination was discontinued when the amount eliminated fell to the average normal value. The data in Table 1 represent the mg. of nicotinamide methochloride eliminated, diminished by the average normal elimination during the same period of the day (based on a 3-day average (Fig. 1)). The figures in the last column for each subject are obtained by multiplying the mg. eliminated by 0.707 to correct for the difference in molecular weight between nicotinamide methochloride and nicotinamide.

The recovery of the methochloride after ingestion of nicotinamide from the normal subject (1) is two to three times greater than that from the diabetic subject (3); the recovery from subject (2) is more or less intermediate. Subject (1) eliminates about 25 % when given 100 mg. or more of the amide; if lower amounts are taken, the relative elimination is less. The same relation exists, although on a lower level, in subject (3); elimination by subject (2) is less consistent. During the first 13 hr. about 80 % of the total nicotinamide methochloride is eliminated irrespective of the amount ingested by all three subjects.

Compared with nicotinamide, nicotinic acid is converted to a smaller extent by subjects (1) and (2) (normals), the relative amount of the methyl-derivative eliminated by subject (3) being almost the same as after ingestion of nicotinamide. The conversion rate for nicotinic acid is much slower in all three subjects than for nicotinamide. The individual elimination curves of nicotinamide methochloride after intake of 100 mg. of nicotinamide and of nicotinic acid for subjects (1) and (3) are shown in Figs. 2 and 3.

After the ingestion of small doses of nikethamide the amount converted in subjects (1) and (2) is less than that for nicotinamide; after doses of 500 mg. the total amount converted is much the same as after similar amounts of nicotinamide. Subject (3)

Table 1. Nicotinamide methochloride eliminated after ingestion of nicotinamide, nicotinic acid, and nikethamide by subjects (1), (2) and (3)

Compound ingested	Dose (mg.)	Subject (1)										Subject (2)										Subject (3)									
		Nicotinamide methochloride eliminated										Nicotinamide methochloride eliminated										Nicotinamide methochloride eliminated									
		(mg.)										(mg.)										(mg.)									
		during (hr.)										during (hr.)										during (hr.)									
		0-13	13-37	37-61	61-85	Total	Total gested)				0-13	13-37	37-61	Total	Total gested)				0-13	13-37	37-61	61-85	Total	Total gested)							
Nicotinamide	50	6-8	—	—	—	6-8	9-6					—	—	—	—	—	—					3-0	—	—	—	3-0	4-2				
	100	28-1	4-0	—	—	32-1	22-7					23-9	—	—	23-9	16-9					8-8	1-9	—	—	10-7	7-6					
	200	72-0	5-4	—	—	77-4	27-4					44-2	—	—	44-2	15-6					24-3	0-7	—	—	25-0	8-8					
	500	146-4	24-3	—	—	170-7	24-1					68-6	5-2	—	73-8	10-9					67-7	5-1	—	—	72-8	10-3					
Nicotinic acid	100	13-4	—	—	—	13-4	9-5					2-9	6-2	—	9-1	8-2					6-4	1-8	—	—	8-2	5-8					
	200	20-1	9-4	5-4	—	34-9	12-3					17-9	3-1	—	21-0	7-4					13-6	10-3	1-2	—	25-1	8-9					
Nikethamide (in doses equiv. to nicotinamide on mol. wt. basis)	100	9-4	3-2	—	—	12-6	8-9					13-6	3-2	—	16-8	11-9					5-9	2-9	—	—	8-8	4-2					
	200	19-8	18-0	—	—	37-8	13-4					18-5	8-7	—	27-2	9-6					13-2	11-1	3-3	—	27-6	9-8					
	500	54-3	85-3	15-6	5-9	161-1	22-8					63-2	46-7	8-7	118-6	16-7					28-7	26-0	5-1	3-5	63-5	9-0					

eliminates about the same percentage of the methylated derivative after ingestion of nicotinamide, nicotinic acid or nikethamide. In all three subjects the speed of elimination after ingestion of nikethamide is, however, considerably reduced and the duration prolonged; only 30-40 % of the total eliminated appears in the first 13 hr. Nicotinic acid-monoethyl-amide is similar in action to nikethamide.

Fig. 2. Nicotinamide methochloride elimination after intake of 100 mg. of nicotinamide — and nicotinic acid ---- by subject (1). Arrows indicate time of ingestion.

Fig. 3. Nicotinamide methochloride elimination after intake of 100 mg. of nicotinamide — and nicotinic acid ---- by subject (3). Arrows indicate time of ingestion.

Fig. 4 shows a 2 days' average elimination curve of nicotinamide methochloride for 11 healthy young airmen after the intake of 100 mg. of nicotinamide. Although values used in the curve are in arbitrary units the curve is of the same shape as that shown in Fig. 2 for the absolute elimination in mg./hr. The maximum elimination in both curves occurs in the second hour.

The elimination of nicotinamide methochloride was also determined after oral and subcutaneous administration of nicotinamide methochloride in subjects (1) and (3). The recovery is given in Table 2.

The influence of continued daily administration of 100 mg. of nicotinamide on the nicotinamide

methochloride elimination was first studied by examining the first morning urine sample. Fig. 5 shows the nicotinamide methochloride elimination from two groups of young airmen (18-20 years)

Fig. 4. Average nicotinamide methochloride elimination of 11 men for 2 days following the daily intake of 100 mg. nicotinamide. Arrow indicates time of ingestion.

living on the same diet and performing the same light physical work. Beginning with the fourth day of the experiment one group received daily a dose of 100 mg. for 7 days; the other group served as control. There is a marked but irregular rise of the

Table 2. Recovery of nicotinamide methochloride after oral or subcutaneous administration in subjects (1) and (3)

Dose (mg.)	Manner of administration	Recovery as % of intake during (hr.)		
		0-1	0-7	0-24
Subject 1				
100	Oral	0.48	11.6	16.6
200	Oral	0.96	9.2	12.5
100	Subcutaneous	42.5	54.5	61.8
Subject 3				
100	Oral	1.3	7.1	12.6
200	Oral	0.93	6.7	12.9
100	Subcutaneous	31.7	50.6	61.8

nicotinamide methochloride output; the level, however, is always higher than that of the controls. The depressions in the curves also occur in the control group and are probably due to extrinsic factors such as food, temperature, work, etc., equally affecting both groups, and therefore not connected with the administered nicotinamide.

Similar results were obtained from two other groups, each of 15 healthy young airmen. One of these groups received each day for 22 days mixed vitamin tablets containing 5 mg. of aneurin, 3 mg. of riboflavin, 50 mg. of nicotinamide, 5 mg. of pyridoxine, and 10 mg. of calcium pantothenate. Both groups were living on the same diet and working under the same conditions. The continued 'saturation' rise was interrupted on the fourteenth

day of dosage for some unknown reason. A return to the former high level occurred a week later.

The effect of repeated ingestion of a daily dose of 100 mg. of nicotinamide on the elimination of the methyl derivative was studied in two groups of 10 and 8 laboratory workers selected at random and living on similar war rations. The group of 10 men

Fig. 5. Nicotinamide methochloride elimination in early morning samples of two groups of 10 men without +---+ and with x---x daily intake of 100 mg. nicotinamide. The arrow indicates the beginning of the nicotinamide ingestion.

received 100 mg. of nicotinamide on the first day, no extra amide for the next 2 days, and 100 mg. on each of the following 4 days. The urine was collected during 7 hr. after the administration of the first, fourth and seventh days of the experiment and the nicotinamide methochloride content was determined. The group of eight men received 100 mg. nicotinamide a day for 5 consecutive days. The urine was collected for the 7 hr. intervals after the administration on the first, third and fifth days of the experiment and the methochloride estimated. The average elimination values in the first group were 13.6 mg. on the first, 14.9 on the fourth and 19.1 mg. on the seventh day. In the second group the values were 15.2 mg. on the first, 21.1 mg. on the third, and 22.3 mg. on the fifth day. These values have not been corrected by subtracting the normal daily excretion of the methyl derivative, which was unknown.

DISCUSSION

The shape of the daily normal curves seems to be consistent for the same individual at different times and indeed for different individuals. These fluctuations are still apparent on the second day after the ingestion of 500 mg. doses of nikethamide or nicotinamide, although the absolute height of the elimination is much greater. The relative height of the maxima in the normal curves varies considerably from day to day in the same individual and even more from person to person. The total daily elimina-

tion from our three subjects varied from 2 to 8 mg./day. These figures seem to be higher than those reported by Sarett (1943) for F_2 and by Wang & Kodicek (1943) for the total nicotinamide derivatives estimated in the urine. It seems probable that most of the so-called 'trigonelline' in the urine is nicotinamide methochloride, and that the trigonelline actually present is mainly due to that present in the diet. Trigonelline treated with alkaline *iso*-butanol does show a faint fluorescence, but the fluorescence efficiency in the 366μ line is so low as to be negligible in the assay. The ingestion of trigonelline showed no increase in the urinary output of nicotinamide methochloride.

It is not astonishing that the intake of the usual food has no immediate effect on the nicotinamide methochloride elimination, since the concentration of nicotinamide in the food now available is too low to produce a marked rise in the output. If the normal daily methochloride elimination is about 7.5 mg./day and if one assumes that approximately 15 % of the ingested nicotinamide is eliminated as the methyl derivative (the average elimination after nicotinamide doses), this would indicate the presence of more than 40 mg. of nicotinamide or nicotinic acid in the food ingested each day. This value is almost inconceivably high if one is to accept the values for the content of nicotinamide and similar compounds in the foods reported by numerous investigators. This suggests an extra dietary source of either nicotinamide, nicotinic acid or nicotinamide methochloride. It could be due to bacterial formation and the subsequent release of nicotinamide or nicotinic acid in the gut, as shown for aneurin by Najjar & Holt (1943) or less probably to the synthesis of one or all of these compounds in the human body. Experiments to study this question are in progress.

The effect of alcoholic drinks, a short increase in elimination followed by a lasting decrease, might be explained by the action of ethanol on the intestinal flora or by interference with the mechanism of methylation in the liver (Perlzweig, Bernheim & Bernheim, 1943). The amount of nicotinamide methochloride eliminated is probably influenced by physical exertion, and this must be taken into account in the evaluation of the normal daily output.

The rapid increase in the elimination of nicotinamide methochloride after the ingestion of nicotinamide shows that the methylation process is a rapid one. Only a small proportion of the ingested amide is eliminated as the methyl derivative; the highest proportion (43 %) occurred in subject (1) on the fifth day of a daily 100 mg. nicotinamide dosage. The fate of the remainder is not known. The daily increase in the output after successive doses of the active compounds and the relatively greater proportion eliminated after the higher doses than with

small amounts seems to indicate a certain amount of storage in the body.

The elimination of nicotinamide methochloride depends not only on the amount of nicotinamide ingested but probably on the reserves of 'methyl-donators', i.e. substances that contribute methyl groups as well. On account of the present food restrictions it was impossible to examine this factor.

That still another factor is involved can be seen from the low conversion by the diabetic subject (3) who obtained far more animal protein and therefore more of the methyl donors than the others tested. This suggests a connexion between nicotinamide and carbohydrate metabolism, already described by Göbell (1941) and Neuwahl (1943), who found a reduction of the blood sugar after the intake of nicotinamide and nicotinic acid.

The conversion for subject (3) was much the same for nicotinamide and nicotinic acid, and showed that only the methylation but not the amidation was defective.

The results given in Table 2 show that nicotinamide methochloride is slowly and incompletely absorbed from the gut, and no other conclusion can be drawn from the experiments using oral administration. When given parenterally it is rapidly eliminated in the urine (about 40 % in 1 hr.); none can be found after 24 hr. About 40 % cannot be accounted for. The elimination of 61.8 % in 24 hr. by both subjects (1) and (3), shows that differences observed in the elimination of nicotinamide methochloride after the intake of nicotinamide by these two subjects cannot be due to different rates of destruction.

Pyridoxine had no effect on the elimination of nicotinamide methochloride; Single & Sydenstricker (1941) reported the elimination of another fluorescent substance after the ingestion of pyridoxine, which we were unable to confirm.

The physiological importance of the nicotinamide methochloride elimination in urine is not yet fully established. It is certain that the height of the elimination depends on the direct or indirect intake and on the storage of nicotinamide and related compounds, on the amounts of these compounds used up by the body, and probably on the amount of methyl-donators available in the body and on the efficiency of the methylating mechanism.

SUMMARY

1. The total daily urinary elimination of nicotinamide methochloride by man was determined and found to vary individually and in the same person at different times within the range of 2 and 8 mg. in 24 hr.

2. Ingested nicotinamide increases the elimination of nicotinamide methochloride. The study of the elimination suggests the occurrence of storage

and saturation of nicotinamide. Nicotinic acid, nikethamide and nicotinic acid-mono-ethyl-amide have a similar but quantitatively different action.

3. The hourly fluctuations of the nicotinamide methochloride elimination were studied and found to be identical in different persons and in the same person under different conditions.

4. The effect of a number of factors (food, alcohol, work) influencing the elimination of nicotinamide methochloride was studied.

5. The height of the nicotinamide methochloride elimination is determined by the intake of nicotinamide and related compounds and their use by the body, the presence of methyl-donators and the efficiency of the methylating mechanism.

6. The relation of nicotinamide methochloride eliminated in the urine to the nicotinamide ingested with the food indicates the presence of an extra dietary source of nicotinamide.

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Investigations on the Activity of the Histaminase in Normal and Toxaemic Pregnancy

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In some recent papers I have suggested that histaminase may play an important part in the metabolic changes occurring in normal and toxaemic pregnancy (Kapeller-Adler, 1941 *a, b, c*; Kapeller-Adler & Adler, 1943). From the experimental data obtained it has been assumed that histamine might be formed, in the metabolism of pregnant women, from the histidine present in large amounts throughout gestation, by the activity of histidine decarboxylase. In normal pregnancy most of the histamine formed is presumably destroyed by histaminase so that only traces escape destruction, to be excreted in the urine. It has further been suggested that in mild cases of pre-eclamptic toxaemia the activity of the histaminase may be impaired, and that more histamine may thus escape and be available to cause various kinds of damage.

Much of it, however, seems to be eliminated in the urine. In severe cases of pre-eclamptic toxaemia and in eclampsia a condition may arise where the activity of the histidine decarboxylase may be increased, whereas that of the histaminase may be completely inhibited. Thus much histidine would be converted into histamine which, not being destroyed by histaminase, would cause considerable damage, especially to the liver and kidneys. The latter would then lose the ability to excrete histidine and histamine, which would be completely retained in the tissues. The object of the present paper was to obtain evidence of the role played by the histaminase in normal and toxaemic pregnancy.

Marcou (1937) reported that the blood in pregnancy has an extraordinarily high activity in destroying histamine. Other investigators (Werle &